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# RESISTANCE OF *BOTRYTIS CINEREA* TO VINCLOZOLIN, IPRODIONE AND DICLORAN

by

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## ABSTRACT

Isolates of Botrytis cinerea from containerized conifer seedlings from two nurseries in the Northern Rocky Mountains were exposed to the dicarboximide fungicides vinclozolin and iprodione and the chlorinated nitroaniline fungicide dicloran to evaluate occurrence and characteristics of resistant strains. The isolates were grown on test media prepared by incorporating the fungicides at various concentrations into potato dextrose agar. In this way, we were able to select B. cinerea strains resistant to increasing fungicide concentrations up to a maximum of 10,000 ug/ml. Resistance to the three fungicides occurred in isolates not previously exposed to the chemicals. Some strains were even resistant to high fungicide concentrations. Genetic stability of these resistant strains was confirmed by growing them in the absence of fungicides. Cross-resistance among the three fungicides was common. Vinclozolin and iprodione generally inhibited spore germination more than mycelial growth; the opposite was true for dicloran. Ramifications of fungicide resistance in disease control are discussed.


## INTRODUCTION

Blight of conifer seedlings caused by the fungus Botrytis cinerea (Fr.) Pers. is a serious disease in many nurseries of western North America (James 1984; McCain 1978). This disease is usually most damaging on containerized seedlings grown in greenhouses (James et al. 1982; James and Genz 1983; McCain and Smith 1978). However, it can also occur on and cause damage to bareroot stock (James 1980; James et al. 1983).

Fungicides have traditionally been used to control Botrytis blight (Maude 1980). However, their effectiveness has decreased because the fungus has developed resistance to some of them (Cooley 1981; Gillman and James 1980; James and Gilligan 1983). Resistance of B. cinerea to the systemic fungicide benomyl was initially reported several years ago (Bollen and Scholten 1971); occurrence of resistant fungal strains to this chemical is now common (Gillman and James 1980; James and Gilligan 1983; Miller and Fletcher 1974; Pepin and MacPherson 1982; Watson and Koons 1973). Botrytis has also exhibited resistance to several other common fungicides including chlorothalonil (Cooley 1981; James and Gilligan 1983), captan (Cooley 1981; Parry and Wood 1959; Pepin and MacPherson 1982), and dicloran (Fritz, Leroux and Gredt 1977; Webster, Ogawa, and Base 1970).

Because of the importance of fungicide resistance, new chemicals initially developed for nonconifer crops were evaluated for Botrytis control (James and Woo 1984). Two of these, vinclozolin[1] (Ronilan®, Ornalin®) and iprodione[2] (Chipco 26019®, Rovral®), effectively controlled B. cinerea in field tests, although vinclozolin exhibited some phytotoxicity

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[1] 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione

[2] 3(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboximide

to containerized western larch seedlings in one test (James and Genz 1983). Unfortunately, past experience has indicated that B. cinerea can rapidly develop resistance to both these fungicides, especially if they are applied continuously at high concentrations (Beever 1983; Katan 1981; Pappas, Cooke and Jordan 1979).

Because new fungicides are needed to combat Botrytis blight in conifer seedling nurseries (James 1984), tests were conducted to evaluate ease of development and natural occurrence of resistance by the fungus to vinclozolin and iprodione. We also tested resistance to dicloran[3], because this fungicide is frequently being used in many nurseries.

## MATERIALS AND METHODS

### Test 1. Resistance of B. cinerea to increasing concentrations of vinclozolin, iprodione, and dicloran.

This test was designed to select isolates of the fungus that could successfully grow on potato dextrose agar (PDA) amended with increasing concentrations of vinclozolin, iprodione, and dicloran. Strains that grew successfully on 5 µg/ml[4] of the fungicides were subjected to successively higher concentrations to a maximum of 10,000 µg/ml. Checks consisted of growing isolates on unamended PDA. All fungicides were wettable powders which were added to liquid autoclaved PDA while at 45-55°C. Petri plates were incubated in the dark at 22°C (standard conditions for all tests) for 6 days, after which colony diameters (linear growth over agar surface) were measured. Fungicide effectiveness was expressed as percent growth inhibition compared to the checks.

Five isolates of B. cinerea were selected for this test. Three isolates (81-33, 82-28, 82-29) were obtained from containerized lodgepole pine seedlings of the Flathead Indian Reservation Greenhouse, Ronan, Montana. These isolates had previously been screened for resistance to several common fungicides (James and Gilligan 1983). The other two isolates (80-29, 80-39) were obtained from containerized lodgepole pine and western larch seedlings, respectively, at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. All isolates were grown on PDA for several months prior to this test.

### Test 2. Resistance of numerous B. cinerea isolates to low concentrations of vinclozolin, iprodione, and dicloran

Twenty-seven isolates of B. cinerea obtained from containerized seedlings at the Flathead Indian Reservation Greenhouse, Ronan, MT or the USDA Forest Service Nursery, Coeur d'Alene, ID were tested for resistance to 10 µg/ml of vinclozolin, iprodione, and dicloran. Isolates were grown for 4 days on PDA at 22°C under cool fluorescent light for 12 hours, then

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[3] 2-6-Dichloro-4-nitroaniline

[4] All concentrations are µg active ingredient of the fungicide and correspond to parts per million.

transferred to petri plates with fungicide-amended PDA. Isolates grown on unamended PDA served as checks. Petri plates were incubated at standard conditions for 6 days, after which linear fungal growth was measured. Growth rates on fungicide-amended PDA were expressed as percent of growth on unamended PDA.

Test 3. Performance of resistant B. cinerea strains (test 2) at higher fungicide concentrations.

Strains of B. cinerea which grew well on PDA amended with 10 µg/ml (test 2) were exposed to higher concentrations of the same fungicide to determine if they were resistant to these higher levels. Twelve of the 27 isolates that were considered resistant to 10 µg/ml were exposed to either 500 µg/ml of dicloran or 5,000 µg/ml of vinclozolin or iprodione. Strains grown on unamended PDA served as checks. Standard incubation procedures were used and linear growth was measured after 6 days. Growth on fungicide-amended PDA was expressed as percent of growth on check plates.

Test 4. Performance of parent and fungicide-resistant strains of B. cinerea (test 1) on high concentrations of vinclozolin and iprodione.

Tests were conducted to determine if resistance of B. cinerea strains exhibited in test 1 was inherent in the parent strains or acquired as they were exposed sequentially to higher fungicide concentrations. Vinclozolin-resistant strains and their parents (unexposed to fungicides) were grown on standard PDA and PDA amended with 7,500 µg/ml and 10,000 µg/ml of vinclozolin. Similar tests were conducted for iprodione-resistant strains at fungicide concentrations of 2,500 µg/ml and 10,000 µg/ml. All petri plates were incubated under standard conditions and linear growth was expressed as mm/day.

Test 5. Stability of resistance of B. cinerea strains to vinclozolin and iprodione.

Strains of B. cinerea selected for resistance to vinclozolin and iprodione (test 1) were evaluated for their ability to remain resistant when maintained in the absence of fungicides. Resistant strains were grown in the absence of fungicides on standard PDA for 4 days, then transferred to PDA amended with 10,000 µg/ml vinclozolin and iprodione. Plates were incubated under standard conditions and linear growth measured after 9 days.

Resistant strains were also maintained in the absence of fungicides on standard PDA for 24 weeks. They were then transferred to PDA amended with 10 µg/ml and 50 µg/ml vinclozolin and iprodione, incubated at standard conditions and measured for linear growth after 15 days.

Test 6. Cross resistance of B. cinerea strains to vinclozolin, iprodione, and dicloran.

Fungicide-resistant strains (test 1) were evaluated for their ability to be cross-resistant to other fungicides. For example, vinclozolin-resistant strains were evaluated for their ability to grow on PDA amended with iprodione and dicloran. Resistant strains were exposed to 10 and 1,000 µg/ml of each test fungicide, incubated at standard conditions and linear growth measured after 7 days.

Test 7. Effects of vinclozolin, iprodione, and dicloran on conidial germination of B. cinerea.

Effects of fungicides on conidial germination were evaluated for the five B. cinerea isolates tested in test 1. Spore suspensions were prepared from 14 day-old PDA cultures which were grown under black light to enhance sporulation (Epton and Richmond 1980). Cultures were flooded with sterile distilled water and agitated with a fine camel's hair brush. Spore suspensions were filtered through cheesecloth to remove mycelial fragments and adjusted to a concentration of about $1.5 \times 10^6$ spores/ml. About 0.1 ml of each suspension was pipetted onto two petri plates with PDA amended with 5 µg/ml of either vinclozolin, iprodione, or dicloran and spread uniformly over the surface with a sterile glass rod. Plates were incubated at $22^{\circ}C$ in the dark for 24 hours and examined under the microscope (100X) for conidial germination. Two hundred spores of each isolate were randomly examined for germination; percentage germination (emergence of germ tubes) for each isolate on each fungicide was calculated.

## RESULTS

Test 1. From the B. cinerea isolates evaluated, five strains resistant to vinclozolin (80-29 #3, 80-39 #3, 81-33 #1, 81-33 #3, 82-28 #2), four resistant to iprodione (80-29 #1, 80-29 #2, 80-39 #3, 82-28 #1) and one resistant to dicloran (80-29 #2) were selected in a series of exposures to increasingly higher concentrations of the fungicides (table 1). Some resistant strains grew fast and others more slowly on fungicide-amended media (figure 1). A few strains obtained were resistant to as high as 10,000 µg/ml of the test fungicides.

Responses to increasing fungicide concentrations are compared graphically in figures 2, 3, and 4 for strains resistant to vinclozolin, iprodione, and dicloran, respectively. For most strains, percent growth inhibition increased with increasing fungicide concentration. However, several strains were less inhibited as fungicide concentration increased from 5 to 100 µg/ml. This indicates that these resistant strains were able to grow faster at increasing concentrations of fungicides, at least until concentrations exceeded 100 µg/ml. However, above this concentration their growth became increasingly inhibited.

Table 1.—Growth responses of selected isolates of *Botrytis cinerea* to increasing concentrations of vinclozolin, iprodione, and dicloran *in vitro* (test 1).[1]

ISOLATES[2]

| Fung. | 80-29 | | | 80-30 | | | 81-33 | | | 82-28 | | | 82-29 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. | Vinclo. | Ipro. | Diclo. | Vinclo. | Ipro. | Diclo. | Vinclo. | Ipro. | Diclo. | Vinclo. | Ipro. | Diclo. | Vinclo. | Ipro. | Diclo. |
| (µg/ml) | 1 2 3 | 1 2 3 | 1 2 2 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |
| 5 | 3 2 30 | 60 28 5 | 5 5 9 | 0 0 36 | 0 4 60 | 4 4 4 | 68 9 49 | 4 5 8 | 8 5 8 | 0 30 0 | 70 7 5 | 15 4 4 | 0 0 0 | 4 8 4 | 3 3 5 |
| 10 | 0 0 81 | 71 50 0 | 0 4 0 | - - 71 | - 0 58 | 0 0 0 | 100 - 61 | 0 0 0 | 0 0 0 | - 83 - | 71 0 0 | 0 0 5 | - - - | 0 0 0 | 0 0 0 |
| 25 | - - 94 | 77 61 - | - 61 - | - - 77 | - - 65 | - - - | 81 - 77 | - - - | - - - | - 81 - | 77 - - | - - 8 | - - - | - - - | - - - |
| 50 | - - 103 | 85 27 - | - 46 - | - - 64 | - - 58 | - - - | 119 - 85 | - - - | - - - | - 97 - | 73 - - | - - 0 | - - - | - - - | - - - |
| 100 | - - 100 | 73 35 - | - 43 - | - - 70 | - - 61 | - - - | 106 - 85 | - - - | - - - | - 88 - | 67 - - | - - - | - - - | - - - | - - - |
| 500 | - - 64 | 61 24 - | - 22 - | - - 49 | - - 52 | - - - | 39 - 73 | - - - | - - - | - 49 - | 52 - - | - - - | - - - | - - - | - - - |
| 1,000 | - - 40 | 33 28 - | - 21 - | - - 40 | - - 43 | - - - | 30 - 62 | - - - | - - - | - 32 - | 45 - - | - - - | - - - | - - - | - - - |
| 2,500 | - - 18 | 21 24 - | - 34 - | - - 33 | - - 32 | - - - | 24 - 30 | - - - | - - - | - 12 - | 28 - | - - | - - - | - - - | - - - |
| 5,000 | - - 14 | 16 13 - | - 3 - | - - 23 | - - 22 | - - - | 23 - 22 | - - - | - - - | - 25 - | 20 - | - - | - - - | - - - | - - - |
| 7,500 | - - 12 | 16 12 - | - - - | - - 30 | - - 24 | - - - | 24 - 33 | - - - | - - - | - 23 - | 14 - | - - | - - - | - - - | - - - |
| 10,000 | - - 9 | 16 10 - | - - - | - - 26 | - - 27 | - - - | 24 - 30 | - - - | - - - | - 21 - | 22 - | - - | - - - | - - - | - - - |

[1]Figures in table are linear growth rates on fungicide-amended PDA expressed as percent of check (growth on unamended PDA).

[2]Each isolate was replicated three times on each test fungicide at each concentration.

[3]Not tested at concentrations above 5,000 µg/ml.

Figure 1.--Growth of Botrytis cinerea strains exhibiting resistance to iprodione (5 µg/ml) after 9 days at 22°C.

Test 2. Of 27 B. cinerea isolates obtained from different conifer host species, five were resistant to vinclozolin and four were resistant to either iprodione or dicloran. Ten isolates exhibited slight resistance, i.e., they grew slightly on PDA amended with 10 µg/ml of the fungicide, and five exhibited some degree of resistance to two or more of the fungicides (table 2). None of these isolates had previously been exposed to any of these fungicides.

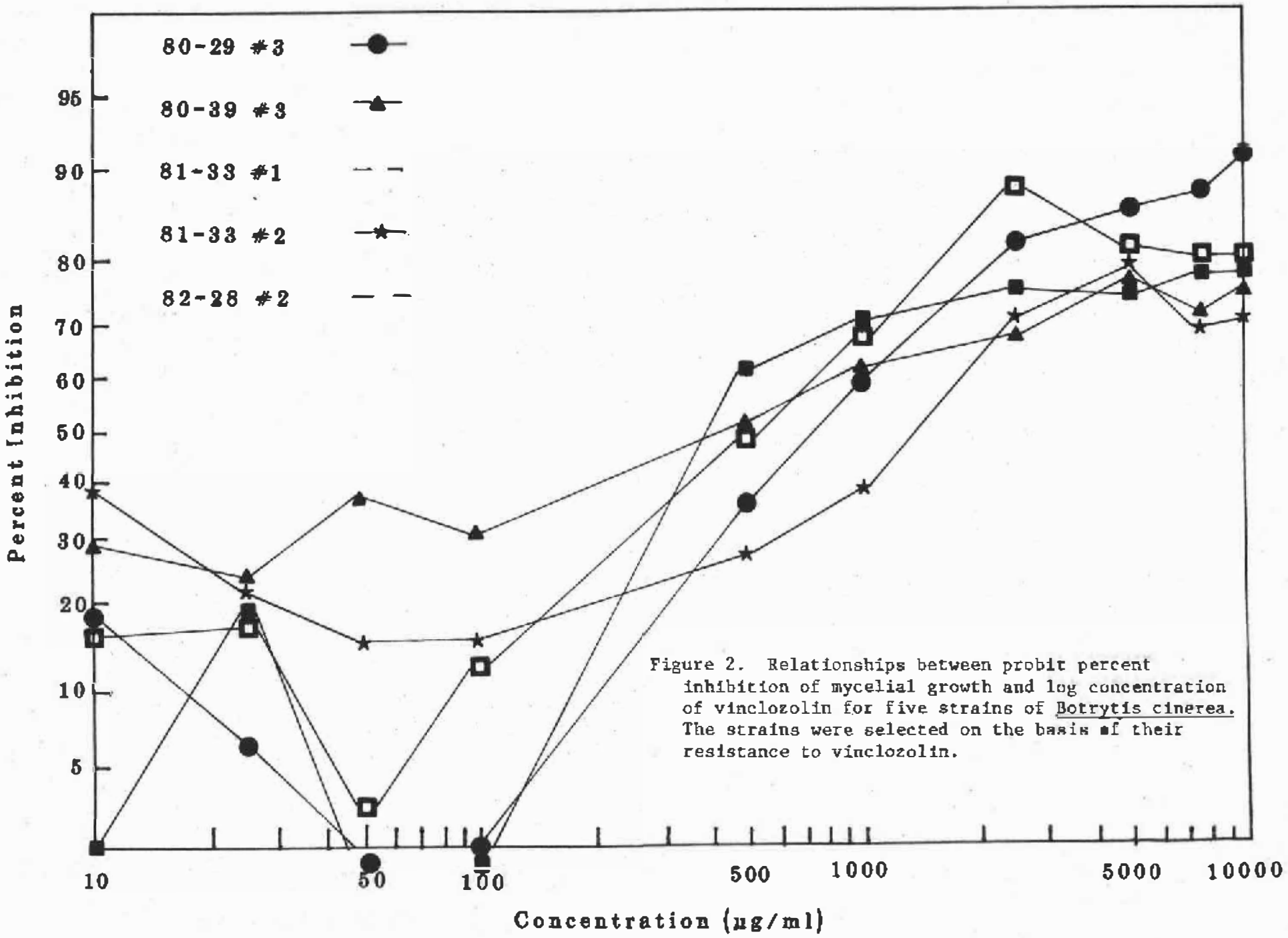


Figure 2. Relationships between probit percent inhibition of mycelial growth and log concentration of vinclozolin for five strains of Botrytis cinerea. The strains were selected on the basis of their resistance to vinclozolin.

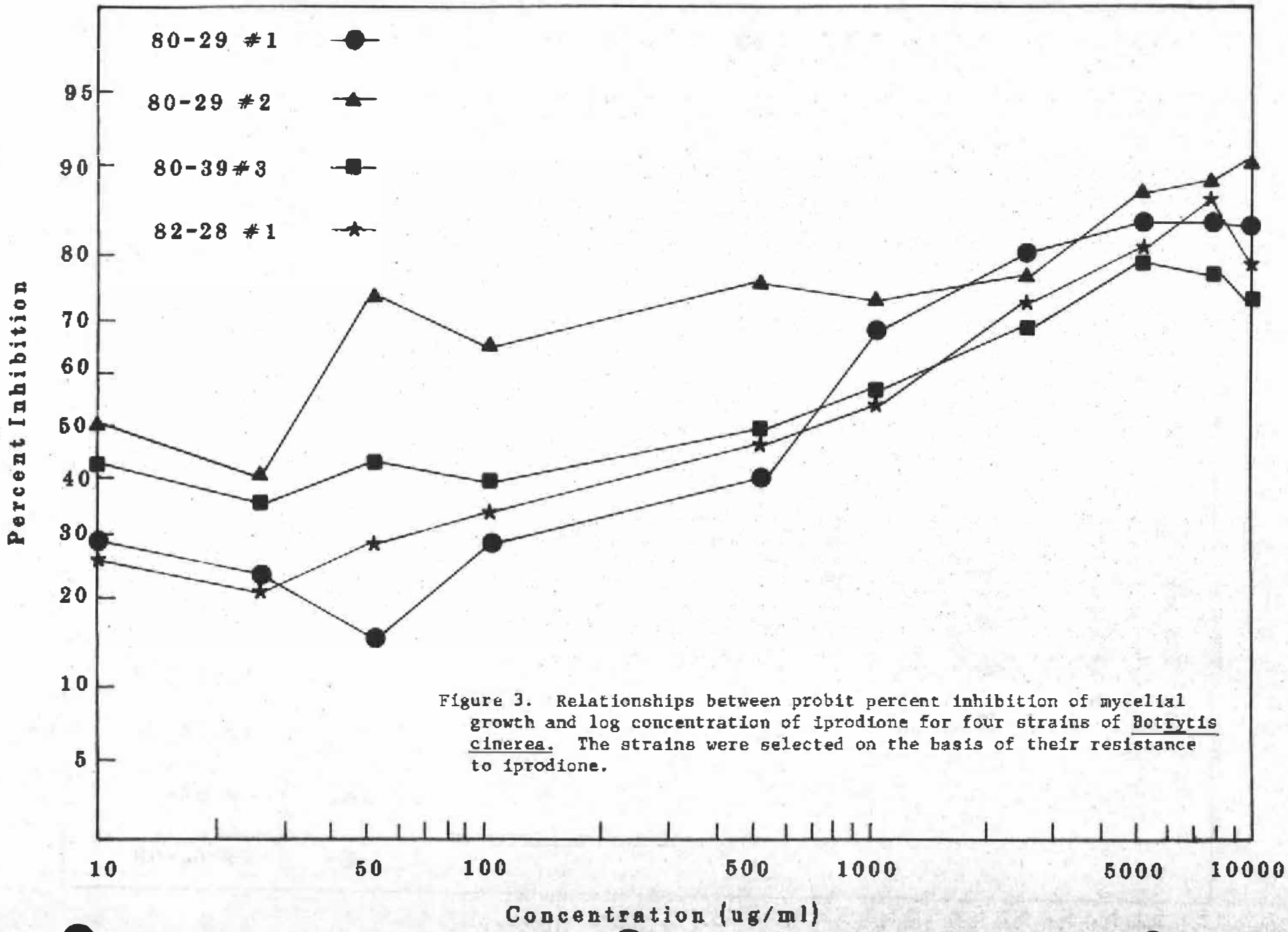


Figure 3. Relationships between probit percent inhibition of mycelial growth and log concentration of iprodione for four strains of Botrytis cinerea. The strains were selected on the basis of their resistance to iprodione.

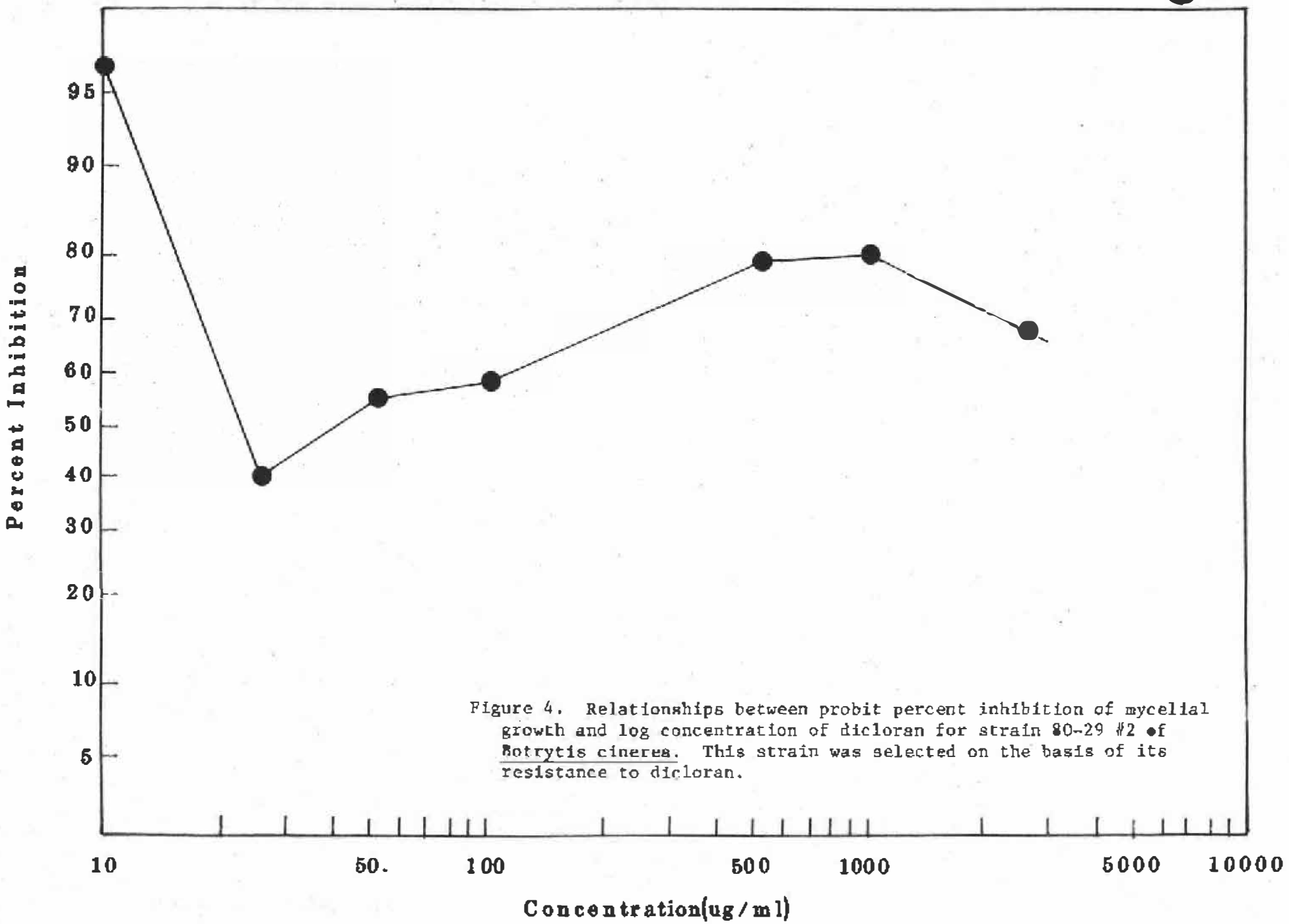


Figure 4. Relationships between probit percent inhibition of mycelial growth and log concentration of dicloran for strain 80-29 #2 of Botrytis cinerea. This strain was selected on the basis of its resistance to dicloran.

Table 2.--Growth of selected isolates of Botrytis cinerea on PDA amended with 10 µg/ml vinclozolin, iprodione, and dicloran (test 2).[1]

| Isolate[2] | Host[3] | Location[4] | Vinclozolin | Iprodione | Dicloran |
|---|---|---|---|---|---|
| 80-14 | WL | CDA | 0 | 0 | 0 |
| 80-21 | WL | CDA | 0 | 0 | 0 |
| 80-22 | LP | CDA | 0 | 1 | 2 |
| 80-31 | PP | CDA | 0 | 0 | 0 |
| 80-32 | ES | CDA | 0 | 0 | 0 |
| 80-38 | WL | CDA | 0 | 0 | 0 |
| 80-40 | WL | CDA | 32 | 0 | 0 |
| 80-41 | LP | CDA | 0 | 0 | 0 |
| 82-24 | LP | BIA | 2 | 0 | 2 |
| 82-25 | LP | BIA | 0 | 57 | 0 |
| 82-26 | LP | BIA | 0 | 0 | 0 |
| 82-27 | LP | BIA | 5 | 32 | 13 |
| 82-20 | LP | BIA | 0 | 0 | 0 |
| 82-31 | LP | BIA | 0 | 0 | 0 |
| 83-2A | ES | CDA | 0 | 0 | 0 |
| 83-2B | ES | CDA | 0 | 0 | 5 |
| 83-2D | ES | CDA | 0 | 0 | 0 |
| 83-2E | ES | CDA | 0 | 0 | 0 |
| 83-2F | ES | CDA | 0 | 0 | 14 |
| 83-2G | ES | CDA | 16 | 0 | 33 |
| 83-2H | ES | CDA | 0 | 0 | 6 |
| 83-2I | ES | CDA | 10 | 0 | 6 |
| 83-2J | ES | CDA | 0 | 0 | 16 |
| 83-2K | ES | CDA | 50 | 16 | 0 |
| 83-2L | ES | CDA | 0 | 0 | 5 |
| 83-2M | ES | CDA | 0 | 63 | 3 |
| 83-20 | ES | CDA | 59 | 0 | 0 |
| Isolates slightly resistant[5] | | | 2 (7.4%) | 1 (3.7%) | 7 (25.9%) |
| Isolates resistant[6] | | | 5 (18.5%) | 4 (14.8%) | 4 (14.8%) |

[1]Figures in table are linear growth rates on fungicide-amended PDA expressed as percent of check (growth on unamended PDA).

[2]First number of each isolate refers to the year it was collected and placed on artificial media.

[3]All hosts were containerized seedlings:
WL = western larch
LP = lodgepole pine
PP = ponderosa pine
ES = Engelmann spruce

[4]CDA = USDA Forest Service Nursery, Coeur d'Alene, Idaho.
BIA = Bureau of Indian Affairs Nursery, Ronan, Montana.

[5]Isolates with some, but less than 10 percent growth on fungicide-amended media.

[6]Isolates with 10 percent growth or greater on fungicide-amended media.

Test 3. Those B. cinerea strains from test 2 which exhibited resistance to low fungicide concentrations (10 ug/ml) were also resistant to higher concentrations of the same fungicides (table 3). The dicarboximide-resistant strains were resistant to 5,000 µg/ml of the fungicides without having been sequentially exposed to increasing fungicide concentrations. Dicloran-resistant strains grew well at fungicide concentrations of 500 µg/ml, indicating that strains resistant to low fungicide concentrations were likewise resistant to higher concentrations.

Table 3.--Growth of isolates of Botrytis cinerea resistant to 10 µg/ml of vinclozolin, iprodione, and dicloran from test 2 on PDA amended with high concentrations of fungicides (test 3).[1]

| Isolate | Fungicide | Concentration (µg/ml) | Percent growth |
|---|---|---|---|
| 80-40 | vinclozolin | 5,000 | 13 |
| 82-25 | iprodione | 5,000 | 13 |
| 82-27 | iprodione | 5,000 | 19 |
| 82-27 | dicloran | 500 | 10 |
| 83-2F | dicloran | 500 | 34 |
| 83-2G | vinclozolin | 5,000 | 33 |
| 83-26 | dicloran | 500 | 31 |
| 83-2J | dicloran | 500 | 21 |
| 83-2K | iprodione | 5,000 | 38 |
| 83-2K | vinclozolin | 5,000 | 22 |
| 83-2M | iprodione | 5,000 | 33 |
| 83-2O | vinclozolin | 5,000 | 14 |

[1] Figures in table are linear growth rates on fungicide-amended PDA expressed as percent of check (growth on unamended PDA). Tested isolates were considered tolerant to 10 µg/ml of the fungicides (see table 2).

Test 4. Parent isolates (not exposed to fungicides) of fungicide-resistant B. cinerea strains selected in test 1 did not exhibit resistance at high fungicide concentrations (table 4; figure 5). In every case, strains resistant to vinclozolin or iprodione grew well at high fungicide concentrations, but parent isolates failed to grow. The process of selecting isolates for fungicide resistance through a series of exposures to increasingly higher concentrations apparently changed their genetic makeup sufficiently to affect their fungicide sensitivity.

Table 4.--Comparisons of linear growth of parent and fungicide-resistant strains of *Botrytis cinerea* on PDA and PDA amended with high concentrations of vinclozolin and iprodione (test 4).

| Isolate | designation[1] | Linear growth rate (mm/day) PDA | vinclozolin concentration (µg/ml) 7,500 | 10,000 |
|---|---|---|---|---|
| 80-29 | Parent | 9.5 | 0 | 0 |
| 80-29 | Resistant #3 | 5.7 | 1.1 | 1.8 |
| 80-39 | Parent | 8.5 | 0 | 0 |
| 80-39 | Resistant #3 | 6.0 | 1.0 | 1.0 |
| 81-33 | Parent | 7.7 | 0 | 0 |
| 81-33 | Resistant #1 | 6.5 | 1.5 | 1.4 |
| 81-33 | Resistant #3 | 7.5 | 2.5 | 2.1 |
| 82-28 | Parent | 7.0 | 0 | 0 |
| 82-28 | Resistant #2 | 6.0 | 0.9 | 0.8 |
| | | | Iprodione concentration (µg/ml) 2,500 | 10,000 |
| 80-29 | Parent | 9.2 | 0 | 0 |
| 80-29 | Resistant #1 | 9.0 | 1.8 | 1.1 |
| 80-29 | Resistant #2 | 1.5 | 1.1 | 0.8 |
| 80-39 | Parent | 10.7 | 0 | 0 |
| 80-39 | Resistant #3 | 5.7 | 2.2 | 1.5 |
| 82-28 | Parent | 6.5 | 0 | 0 |
| 82-28 | Resistant #1 | 5.2 | 1.7 | 1.2 |

[1]Parent isolates were not previously exposed to fungicides. Resistant isolates were obtained by exposing parent isolates to a series of increasing fungicides concentrations (test 1 - see table 1).

Figure 5.--Comparative sensitivity of resistant strains and parent strains of B. cinerea isolate 80-39 to 10,000 µg/ml and 2,500 µg/ml iprodione. Growth at the top of the plates is that of iprodione-resistant strains after 11 days at 22°C; the bottom of each plate was inoculated with the parent strains which failed to grow.

Test 5. Resistance to vinclozolin and iprodione was generally stable for B. cinerea in the absence of fungicide exposure (table 5). Vinclozolin-resistant strains were still resistant to high concentrations of the fungicide after being grown for 4 days on standard PDA. These strains were also resistant to low vinclozolin concentrations after 24 weeks, but most were not resistant to high fungicide concentrations after this time. All iprodione-resistant strains remained resistant to high fungicide concentrations after being grown at least 24 weeks in the absence of the chemical.

Table 5.--Stability of resistance of strains of Botrytis cinerea to vinclozolin and iprodione (test 5).

| Isolate designation[1] | | Linear growth rate (mm/day) Time grown on PDA | | |
|---|---|---|---|---|
| | | 4 days[2] | 24 weeks[3] | |
| | | | A | B |
| Vinclozolin resistant | | | | |
| 80-29 | Resistant #3 | 0.8 | 0.4 | 0 |
| 80-39 | Resistant #3 | 0.7 | 0.3 | 0 |
| 81-33 | Resistant #1 | 1.0 | 0.6 | 0.8 |
| 81-33 | Resistant #3 | 1.2 | 1.2 | 0 |
| 82-28 | Resistant #2 | 1.1 | 0.6 | 0 |
| Iprodione resistant | | | | |
| 80-29 | Resistant #1 | 0.6 | 1.2 | 0.4 |
| 80-29 | Resistant #2 | 0.6 | 0.1 | 0.1 |
| 80-39 | Resistant #3 | 1.4 | 0.5 | 2.0 |
| 82-28 | Resistant #1 | 0.7 | 0.7 | 0.9 |

[1] Resistant strains were obtained by exposing parent isolates to a series of increasing fungicide concentrations (test 1 - see table 1).

[2] Strains were grown on standard PDA for 4 days, then transferred to PDA amended with 10,000 µg/ml vinclozolin or iprodione; linear growth measured after 9 days.

[3] Isolates were grown on standard PDA for 24 weeks, then transferred to PDA amended with 10 µg/ml (A) or 50 µg/ml (B) vinclozolin or iprodione; linear growth measured after 15 days.

Test 6. Most fungicide resistant strains of B. cinerea selected in test 1 exhibited cross resistance to the other two fungicides (table 6). Vinclozolin-resistant strains were also resistant to iprodione and dicloran at both low and high fungicide concentrations. Likewise, iprodione-resistant strains were resistant to vinclozolin and dicloran. However, the one dicloran-resistant strain tested was not resistant to vinclozolin and was only resistant to iprodione at low fungicide concentrations (10 µg/ml).

Table 6.--Cross resistance of strains of Botrytis cinerea to vinclozolin, iprodione, and dicloran (test 6).

| Strain designation[1] | | Linear growth rate (mm/day)[2] | | | |
|---|---|---|---|---|---|
| | | Iprodione concentration (µg/ml) | | Dicloran concentration (µg/ml) | |
| Vinclozolin | resistant | 10 | 1,000 | 10 | 1,000 |
| 80-29 | Resistant #3 | 6.1 | 2.9 | 6.1 | 2.4 |
| 80-39 | Resistant #3 | 5.0 | 4.3 | 2.9 | 2.9 |
| 81-33 | Resistant #1 | 5.0 | 2.3 | 4.3 | 2.7 |
| 81-33 | Resistant #3 | 6.1 | 3.6 | 3.1 | 2.9 |
| 82-28 | Resistant #2 | 5.4 | 1.7 | 2..6 | 2.7 |
| | | Vinclozolin concentration (µg/ml) | | Dicloran concentration (µg/ml) | |
| Iprodione | resistant | 10 | 1,000 | 10 | 1,000 |
| 80-29 | Resistant #1 | 6.1 | 2.6 | 6.1 | 2.7 |
| 80-29 | Resistant #2 | 2.1 | 1.3 | 2.7 | 1.6 |
| 80-39 | Resistant #3 | 6.1 | 1.6 | 3.6 | 2.1 |
| 82-28 | Resistant #1 | 4.4 | 2.3 | 3.6 | 2.3 |
| | | Vinclozolin concentration (µg/ml) | | Iprodione concentration (µg/ml) | |
| Dicloran | resistant | 10 | 1,000 | 10 | 1,000 |
| 80-29 | Resistant #2 | 0 | 0 | 3.6 | 0 |

[1]Resistant strains were obtained by exposing parent isolates to a series of increasing fungicide concentrations (test 1 - see table 1).
[2]Linear growth on PDA amended with test fungicides measured after 7 days.

Test 7. Vinclozolin and iprodione at low concentrations (5 µg/ml) effectively prevented conidial germination of the five B. cinerea isolates tested (table 7). Only a few conidia of one isolate (82-29) germinated on vinclozolin-amended PDA; germ tubes from these conidia were enlarged and lysed shortly after emergence.

Dicloran was much less effective in preventing conidial germination in only two of the isolates tested (80-29 and 81-33). Germ tubes of conidia exposed to dicloran were short, but otherwise appeared normal.

Table 7.--Effects of vinclozolin, iprodione, and dicloran on conidial germination of selected isolates of Botrytis cinerea (test 7).[1]

| Isolate | Fungicide (5 µg/ml) Vinclozolin[2] | Iprodione | Dicloran[3] |
|---|---|---|---|
| 80-29 | 0 | 0 | 6.9 |
| 80-39 | 0 | 0 | 43.8 |
| 81-33 | 0 | 0 | 8.9 |
| 82-28 | 0 | 0 | 50.4 |
| 82-29 | 0.5[4] | 0 | 41.4 |

[1]Figures in table are percent germination of 200 randomly selected conidia after being incubated for 24 hours at 22°C in the dark.
[2]Spores lysed on fungicide-amended media.
[3]Germ tubes were short, but otherwise appeared normal.
[4]Germ tubes enlarged and lysed.

## DISCUSSION

Strains of B. cinerea exhibiting resistance to the dicarboximide fungicides vinclozolin and iprodione as well as the chlorinated nitroaniline fungicide dicloran were easily selected in vitro. Resistance occurred despite the fact that none of the isolates tested had previously been exposed to these fungicides. Several strains were resistant to high fungicide concentrations as well as low fungicide concentrations.

Botrytis cinerea strains that are resistant to many different fungicides are easily selected in vitro (Beever 1983; Davis and Dennis 1981; Guillino and Garibaldi 1981; Leroux, Gredt and Fritz 1981; Webster, Ogawa and Bose 1970). However, such selection does not necessarily mean that these fungicides will be ineffective in controlling the disease under greenhouse or field conditions (Staub et al. 1979). Some reports indicate that resistant fungal strains are less able to survive (Beever 1982) or less pathogenic (Guillino and Garibaldi 1979; Leroux, Gredt and Fritz 1981) than sensitive strains and never reach sufficient populations under natural conditions to do much damage. On the other hand, several reports (Davis and Dennis 1981; Leroux, Fritz and Gredt 1977; Maraite et al. 1980; Pappas, Cooke and Jordan 1979) indicate that fungicide resistant strains are just as pathogenic as sensitive strains. If disease intensity increases despite repeated fungicide applications, presence of resistant fungal strains should be suspected (James and Gilligan 1982; Staub and Sozzi 1984).

Our results indicate that stable genetic changes most likely occur when B. cinerea strains develop fungicide resistance because these resistant strains often remain resistant even in the absence of the fungicide. Stability of resistance in the absence of intensive selection pressure exerted by fungicides has been previously reported (Davis and Dennis 1981), although under field conditions resistant B. cinerea strains have been shown to become less common and finally disappear if the resistant fungicide is excluded (Guillino and Garibaldi 1981). However, reintroduction of the particular fungicide may result in renewed development of resistant fungal strains, particularly if repeated chemical applications are made (Delp 1980; Staub and Sozzi 1984).

In our tests, vinclozolin and iprodione were more effective in restricting conidial germination than limiting hyphal growth of B. cinerea. However, some other reports (Davis and Dennis 1981; Reilly and Lamoreaux 1981) indicate that mycelial growth is much more sensitive to these fungicides than spore germination. Several investigators agree that both vinclozolin and iprodione are very effective in restricting B. cinerea spore germination (Fritz, Leroux and Gredt 1977; Katon 1981; Leroux, Fritz and Gredt 1977). Both these fungicides are generally protective (Rowe 1983; Watkins 1983), although limited systemic action has been reported for iprodione (Danneberger and Vargas 1982). This means that the fungicides must inhibit spore germination to be effective since they have little or no therapeutic value once infection has occurred (Powell 1982; Rowe 1983). Our results indicated that dicloran was more effective restricting mycelial growth of B. cinerea than inhibiting spore germination. Other reports (Esuruoso, Price and Wood 1968; Ritchie 1981, Webster, Ogawa and Bose 1970) indicated that this fungicide effectively inhibits spore germination and mycelium growth, and those spores that do germinate produce shortened, abnormal germ tubes (Sharples 1961). We also found shortened germ tubes on germinated spores, but did not evaluate effects on infection processes. By delaying spore germination (Sharples 1961), dicloran may be particularly effective as a protectant fungicide if germ tubes do not penetrate host tissues prior to lysis.

We found that B. cinerea strains resistant to either vinclozolin, iprodione, or dicloran were usually resistant to the other two fungicides. Cross-resistance among these three fungicides has been previously reported for B. cinerea (Fritz, Leroux and Gredt 1977; Leroux, Gredt and Fritz 1981; Mariate et al. 1980; Panagiotaku and Malathrakis 1981; Pappas, Cooke and Jordan 1979) and other pathogens including Fusarium nivale (Fr.) Ces. (Chastagner and Vassey) and Alternaria alternata (Fr.) Keissler (McPhee 1980). Botrytis strains resistant to dicarboximides and dicloran have been shown, in some cases, to be resistant to benzimidazoles (Beever 1982; Panagiotaku and Malathrokis 1981). However, other reports (Eichhorn and Lorenz 1978; Leroux, Fritz and Gredt 1977) have indicated no correlation in resistance between benzimidazoles and dicarboximides or dicloran. Problems of cross resistance to several commonly used fungicides reduces the available options for growers to successfully control Botrytis blight.

Our tests confirm that B. cinerea is capable of developing resistance to many fungicides if enough selection pressure is exerted on the organism. Selection pressures can be reduced by using several different fungicides alternately or in combination (Edgington et al. 1980; Staub and Sozzi 1984), particularly if alternative chemicals have different modes of action. For example, using fungicides with wide spectrum toxicity such as captan in conjunction with more site specific chemicals is often effective. However, B. cinerea can even develop resistance to captan if selection pressures are strong enough (Cooley 1981; James and Gilligan 1983; Parry and Wood 1959). Therefore, care must be taken to reduce excessive pressures on resident pathogen populations to develop resistance. Along with altering or combining different fungicides, selection pressures can be reduced by maintaining low inoculum levels through periodic sanitation practices, especially between greenhouse crops, and reducing chances of infection by providing proper air circulation or addition of drying agents to irrigation water (James 1984).

Past work has indicated that vinclozolin, iprodione, and dicloran can each effectively control Botrytis blight (McCain 1978; James and Woo 1984; Powell 1982; Rowe 1983; Watkins 1983), especially if applied in combination with other fungicides. However, since Botrytis has the capacity to quickly develop resistance to all these fungicides, care must be taken to ensure that resistant pathogen populations do not become dominant.

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